

## ON THE INCIDENCE AND DISTRIBUTION OF CLONORCHIS METACERCARIAE IN FISH INTERMEDIATE HOSTS IN TAIWAN

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### ABSTRACT

Two thousand seven hundred and sixty four specimens of fresh-water fishes belonging to 11 species were examined for Clonorchis metacercariae. Four species, *i.e.*, *Pseudorasbora parva* (54.65%), *Hemiculter kneri* (53.84%), *Zacco platypus* (20.55%) and *Carassius auratus* (2.24%), were found positive.

The distribution of cysts within 30 *P. parva* was studied. The result shows 84.30% on muscle, 12.14% on head, 2.91% on all fins, 0.10% on scales, 0.17% on gills and 0.38% on skin.

The average number of Clonorchis cysts harboured by each *P. parva* was 419 in summer and 96 in winter. The study of seasonal variations in the incidence of *Clonorchis sinensis* in *P. parva* caught from Kongkuan-pei (lake) in the suburb of Taipei shows the highest incidence in summer, followed by autumn, spring and winter in that order.

The distribution of Clonorchis cysts in the muscle of 60 *P. parva* and 83 *Z. platypus* was studied. In *Z. platypus*, most of the cysts were found on the ventral aspect of the posterior half of the fish (73.8%) and was especially dense at caudal peduncle (41.02%), while in *P. parva*, the location of the cysts was mostly on dorsal aspect of the pectoral region (40.10%).

*Clonorchis sinensis* was discovered in 1875 by McConnell in the biliary passage of a Chinese carpenter in Calcutta (1). As its name "Oriental liver fluke" implies, the endemic area of the fluke disease is well limited to Asia—China, Japan, Korea, The Phillipines and old Indochina. Kobayashi reported in 1910 that the second intermediate host of *Clonorchis sinensis* was a certain species of fresh-water fish belonging to *Cyprinidae* (2). He also stated that the first intermediate host was an aquatic

snail *Semisulcospira* species. Muto reported in 1918 that the proper first intermediate host was *Bithynia striatula* var. *japonica* Pilsbry (3), and thus the entire life history of the fluke was completely elucidated.

To-day 70 species of fresh-water fishes are incriminated to act as the second intermediate hosts of *Clonorchis sinensis* by 16 authors (3-20).

Studies on *Clonorchis sinensis* in Taiwan were started in 1915 by Ohoi who made survey on the incidence of Clonorchiasis in Taichung area and found 40 among 301 hospitalized patients (13.29%) and 70 out of 541 middle school students (12.94%) to be positive (21). Ohoi also was the first

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person to discover *Ctenopharyngodon idellus* to be an important fish intermediate host of *Clonorchis sinensis* in Taiwan (4), Muto reported *Culticulus kneri* (Kreyenberg) (5), and Chow, *Tilapia mossambica* to act as such (6). Recently Kim and Kuntz added another 11 species and 1 subspecies of fishes to the list (7). Therefore, the known intermediate hosts in Taiwan are increased to 14 species (12 of which belong to *Cyprinidae*) and 1 subspecies.

Studies on the *Clonorchis* metacercariae were carried mainly in Japan and China mainland and very few reports have been made concerning the parasites in Taiwan. The present investigation aims to elucidate the incidence and distribution of *Clonorchis* cysts in fish intermediate hosts in this island. Biological studies as to size, specific gravity, resistance of the parasites to various physical and chemical environments and susceptibility of laboratory animals shall be dealt with in another paper.

#### MATERIALS AND METHODS

Batches of fishes were collected from the snail, *Parafossarulus manchouricus*, prevalent areas in Taiwan: Kongkuan-pei near Taipei, Talin in Chia-i, Liuying in Tainan, Chungchen-hu (lake) in Meinung, Tapei-hu (lake) in Kaohsiung and a few specimens from I-lan. A total of 2,764 fresh-water fishes consisting of 11 species were examined. Each specimen was de-scaled and deboned. The fish muscle was scraped with a scalpel or cut into slices, pressed between 2 glass plates (90×45×3 mm), and searched for *Clonorchis* cysts under the dissecting microscope. The number of cysts found were counted and their distribution in the fish was carefully recorded.

To determine the seasonal variations in the incidence of *Clonorchis sinensis* in fish intermediate hosts, 10 *P. parva* were collected and examined from the same water-body Kongkuan-pei every month for a period of 12 months.

#### RESULTS AND DISCUSSION

##### 1. Incidence of *Clonorchis* cysts in the fish intermediate hosts.

Among 11 species of fishes, totaling 2,764 in numbers, examined, 4 species were found infected: 576 out of 1,054 (54.65%) specimens of *P. parva*, 71 out of 130 (53.84%) *Hemiculter kneri*, 246 out of 1,197 (20.55%) *Z. platypus* and 2 out of 89 (2.24%) *Carassius auratus*. All 4 species belong to *Cyprinidae*. The result is shown in TABLE I.

During the examination, 9 different kinds of metacercariae were found in *P. parva*: *Metorchis taiwanensis*, *Metorchis orientalis*, *Cyathocotyle taiwanensis*, *Metacercariae hasegawai*, *Exorchis oviformis*, *Echinochasmus japonicus*, *Stomnosoma formosanum* (?), *Monorchotrema taihokui* and *Clonorchis sinensis*.

Metacercariae of *Metorchis orientalis* is easily confused with that of *Clonorchis sinensis*. However, the former is larger, measuring up to 160×140 microns, than the cysts of *Clonorchis sinensis* which is 140×100 microns. The cyst wall of *Metorchis orientalis* is 3 times thicker and concretion granule in the excretory bladder is finner than those of *Clonorchis sinensis*. These serve as the important features for differentiation between the 2 species (Fig. 1). Other kinds of metacercariae can be easily differentiated from *Clonorchis sinensis* by their peculiar morphological characteristics.

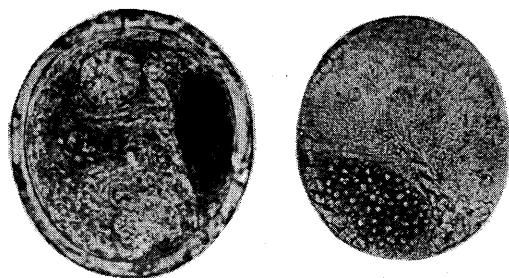


Fig. 1. Metacercariae of *Metorchis orientalis* (right) and *Clonorchis sinensis* (left).

*P. parva* and *H. kneri* are most commonly and heavily parasitized by larvae

TABLE I  
Infection of *Clonorchis sinensis* in 11 species of fishes in Taiwan

Species of fish	Place of origin	No. exam.	No. posit.	Rate %	Month of collection
<i>Zacco platypus</i>	Talin	555	7	1.19	September
	Liuying	397	78	19.70	September
	Tatsu	31	0	0	December
	Nei-hu	23	0	0	November
	Kongkuan-pei	18	10	55.55	November
	Tapei-hu	173	151	87.50	August
		1,197	246	20.55*	
<i>Pseudorasbora parva</i>	Talin	411	2	0.48	October
	Liuying	331	298	90.03	August
	Tapei-hu	29	27	93.10	August
	Nei-hu	12	0	0	December
	Kongkuan-pei	271	249	91.8	May to next April
		10,54	576	54.65*	
<i>Carassius auratus</i>	Coral lake	2	1	50.00	June
	Talin	35	0	0	September
	Nei-hu	14	0	0	November
	Kongkuan-pei	38	1	2.63	March
		89	2	2.24*	
<i>Hemiculter kneri</i>	Tapei-hu	130	71	53.84	August
<i>Tilapia mossambica</i>	Chungchen-hu	34	0	0	June
	Tapei-hu	26	0	0	September
	Nei-hu	114	0	0	October
		174	0	0 *	
<i>Cyprinus carpio</i>	Taipei	37	0	0	January
<i>Parasilurus asotus</i>	Taipei	12	0	0	March
<i>Opsarichthys bidens</i>	Taipei	53	0	0	May, June
<i>Cirrhina moritollera</i>	Coral lake	3	0	0	June
<i>Channa formosana</i>	Taipei	12	0	0	July
<i>Ctenopharyngodon idellus</i>	Miaoli	3	0	0	September

\* The average of the positive rate.

of the liver fluke. *Z. platypus* moderately and *C. auratus* lightly infected. *T. mossambica* from Chungchen-hu was stated to be highly infected by Chow (6). However, in the present study, 34 specimens caught in July from the same lake were examined

and found no infection. Another batches of 26 *T. mossambica* collected from Tapei-hu near Kaohsiung city, where the other species of fishes showed extremely high positive rate, were all negative on examination. All together 174 *T. mossambica*

were examined and not a single one was infected. Positive finding was reported by Kim and Kuntz also, but the incidence was very low. Thus, this fish may not be a favorite intermediate host of *Clonorchis sinensis*. On the other hand, the infection is dependent on the amount and time of the dumping of the infected human excreta into the breeding pond.

2. *Seasonal variations in infection rate of *P. parva* with *Cl. sinensis*.*

The highest rate of 100% was noticed

in the summer months (June, July and August) with 418 metacercariae per fish, next 96.6% in the autumn (September, October and November) with 309 metacercariae per fish. The rate was slightly lower in winter (80.0%) or spring (83.3%). As a matter of fact, the fish was found to be positive through out the year. The number of cysts per fish was below 100 in the cold months when cercariae were not very active. Details are shown in TABLE II.

TABLE II  
*Seasonal variations in infection rate of *Pseudorasbora parva* with *Clonorchis* cysts in northern Taiwan*

Season	Month of collection	No. of fish examined	No. of fish positive	Average no. of cysts per fish
Spring	March	10	8	216
	April	10	8	152
	May	10	9	313
		30	25(83.3%)	Average: 227
Summer	June	10	10	448
	July	10	10	450
	August	10	10	357
		30	30(100%)	Average: 418
Autumn	September	10	10	365
	October	10	9	305
	November	10	10	257
		30	29(96.6%)	Average: 309
Winter	December	10	7	73
	January	10	8	105
	February	10	9	111
		30	24(80.0%)	Average: 96

The infection of *Pseudorasbora parva* with *Clonorchis sinensis* was found to increase suddenly since May. The average number of cysts per fish was only 152 in April but it was as many as 313 in May. As metacercariae of *Clonorchis sinensis* require nearly a month to develop to the maturity, it is clear that the cercariae became active since April when the wea-

ther became warm.

No snail intermediate hosts, *Parafossarulus manchouricus*, were seen around the edge of the pond during the cold months, but they were found mostly under the muddy bottom of the pond. Kim and Kuntz also reported that there was no positive snail intermediate host during the winter (7). However, *P. parva* was found

to harbour the Clonorchis cysts throughout the year and the incidence was as high as 80% even in winter. Thus, it is obvious the metacercariae of *Clonorchis sinensis* can stay within the fish muscle unchanged for more than half a year.

3. *Distribution of Clonorchis cysts in Pseudorasbora parva*

Out of 2,890 cysts harboured in 30 *P. parva*, 2,436 (84.30%) were isolated from the muscle, 351 (12.14%) from the head, 84 (2.91%) from fins, 3 (0.10%) from scales, 5 (0.17%) from gills and 11 (0.38%) from the skin as shown in TABLE III.

Hasegawa in Japan, Komiya in Shanghai and Hsu and Chow in Canton examined the infected *P. parva* for the distribution of Clonorchis cysts. TABLE IV summarizes their results and ours. Some differences were noted between our result and that of Hasegawa's but no significant difference was seen between ours and the other 2 results. It is clear that in case the fish harboured numerous cysts, some might also be found under the scale and on the tail fin, while in fish with fewer cysts, most of the cysts seemed to be located in the fish muscle.

TABLE III  
*Distribution of 2,890 Clonorchis cysts in 30 Pseudorasbora parva*

Part of fish	No. of fish found positive	No. of cysts found	Percentage of cysts
Head	27	351	12.14
Fins	19	84	2.91
Scales	1	3	0.10
Gills	2	5	0.17
Muscle	30	2,436	84.30
Skin	1	11	0.38

TABLE IV  
*Comparison of the distribution of Clonorchis cysts in P. parva obtained by various authors in different localities*

Author, Place No. of fish	Hasegawa, Japan 1		Komiya, Shanghai 1		Hsu & Chow, Canton 1		Huang & Khaw, Taiwan 30		
	Parts of fish	Cysts found							
		No.	%	No.	%	No.	%	No.	%
	Scales	612	17.35	5	0.3	—	—	3	0.10
	Gills	35	0.98	16	0.9	6	1.62	5	0.17
	Tail fin	655	18.57	185	10.8	3	0.86	46	1.38
	Dorsal fin	97	2.18	12	0.8	—	—	18	0.78
	Pectoral fin	48	1.32	11	0.6	—	—	4	0.15
	Ventral fin	68	1.92	3	0.2	—	—	10	0.38
	Anal fin	31	0.89	—	—	—	—	6	0.26
	Muscle	1,933	55.39	1,328	77.4	284	80.94	2,436	84.30
Total no. of cysts found		3,527		1,715		351		2,890	
Average no. of cysts per fish		3,527		1,715		351		96	

4. *Distribution of Clonorchis cysts in the muscle of fish intermediate hosts.*

The distribution of Clonorchis cysts in the muscle was closely examined in 2 species of fish, 60 *P. parva* and 83 *Z. platypus*. In *Z. platypus* most of the cysts were recovered from the posterior half of the fish (87.1%). The soft ventral portion had more encystments than the dorsal

part. They were especially dense at caudal peduncle (41.02%). In *P. parva*, the distribution was different; 40.10% of the cysts were found in dorsal part of the anterior half, just above the lateral line (TABLE V).

Most of the cysts were seen in the superficial layer of the muscle (75%), 16% were at the depth between 1 to 2 mm, and 9% at the depth exceeding 2 mm.

TABLE V  
*Distribution of Clonorchis cysts in the muscle of various parts of Pseudorasbora parva and Zacco platypus*

Location of fish muscle	<i>Zacco platypus</i>		<i>Pseudorasbora parva</i>	
	No. of cysts found	%	No. of cysts found	%
Dorso-pectoral part	22	5.10	2,836	40.10
Dorso-medial part	57	13.28	1,373	19.61
Ventro-pectoral part	33	7.68	939	11.98
Ventro-medial part	141	32.82	755	10.78
Caudal peduncle	176	41.02	1,096	17.53
	429	100.00	6,999	100.00

Regarding the distribution of Clonorchis cysts in the muscle of the fishes, Hsu and Chow reported that 36.2% of the cysts in *P. parva* were found in caudal peduncle, and stated that the caudal region was the most actively moving part of the fish and so offered a better chance for cercariae to encyst (12). This result was in accord with ours in *Z. platypus*. But in *P. parva* of the present study, only 17.53% of the cysts were found in caudal peduncle and 40.10% in the dorsal aspect of pectoral region. Iwata found 29% of the cysts were located in caudal peduncle and 31% in dorsopectoral portion in *P. parva* (22). Thus, the site most densely parasitized by Clonorchis cysts in *P. parva* seems to be the dorso-pectoral portion. The distribution of Clonorchis cysts in *Z. platypus*, on the other hand was quite different from that of *P. parva* in as much as 73.84% of the cysts were found on the ventral aspect of posterior half; and caudal peduncle is the site most densely parasitized (41.02%).

#### CONCLUSION

There are 43 species of fresh-water fishes belonging to the family *Cyprinidae* in Taiwan. 19 of them were reported to act as intermediate hosts of *Clonorchis sinensis* in China, Japan and Korea. In Taiwan, 12 species were found infected and 7 species remain unproven. Further investigation is necessary to clarify this point.

The activity of the snail intermediate host of *Clonorchis sinensis* is evidently influenced by the weather; they become active on the edge of the pond since March and disappear in cold weather (since the end of November). The changes in incidence of Clonorchis cysts in fish intermediate hosts was found to be closely related with the activity of the snail hosts. The relationship between weather and activity of Clonorchis cercariae is an interesting subject.

Most of the Clonorchis cysts were found in fish muscle. The scales and fins

are considered the unfavorable site for their encystment.

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